ISOLATION AND INHIBITORY ACTIVITY OF GABACULINE, A NEW POTENT INHIBITOR OF γ-AMINOBUTYRATE AMINOTRANSFERASE PRODUCED BY A STREPTOMYCES

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1. Introduction

It is well known that GABA plays an inhibitory role in crustacean ans mammalian central nervous system [1,2]. Since GABA does not cross the blood–brain barrier, various inhibitors of GABA aminotransferase which cause an increase in its cerebral level have been introduced into this field of research [3–12]. Most of these inhibitors are very toxic and not specific for GABA aminotransferase.

During our search for new inhibitors of GABA aminotransferase from microbial origin, a potent inhibitor (designated as gabaculine) was found to be present in the culture of a Streptomyces. The present paper describes isolation and inhibitory activity of gabaculine. The results indicate that this new microbial metabolite is a potent, competitive inhibitor of GABA aminotransferase from both beef brain and *Pseudomonas ovalis*, having K_i values of 0.37–0.80 μM.

The physicochemical properties and identification of chemical structure of gabaculine have been reported elsewhere [13].

2. Materials and methods

2.1. *Chemicals*

All chemicals used were of the purest grade commercially available.

Abbreviations: GABA γ-aminobutyric acid, GABA aminotransferase 4-aminobutyrate-2-oxoglutarate aminotransferase (EC 2.6.1.19)

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2.2. *Enzyme preparations*

Beef brain mitochondria and cytosolic fraction were prepared from fresh cerebrum by the method of Waksman et al. [14]. Cell-free extracts, used as a bacterial GABA aminotransferase, were obtained from *Pseudomonas ovalis* as described by Jakoby [15]. For routine experiments, beef brain mitochondria were used as enzyme. Protein was determined by the method of Lowry et al. [16].

2.3. *Enzyme assay*

The reaction mixture (1.0 ml) contained: 50 mM α-ketoglutarate, 100 mM GABA, 0.57 mM pyridoxal phosphate, 100 mM Tris–HCl, pH 8.0 and 10–15 mg protein of beef brain mitochondria or 20–30 μg protein of *Ps. ovalis* cell-free extracts. After incubation of 37°C for 180 min for the former enzyme or 60 min for the latter, the reaction was terminated by addition of 0.3 ml 10% sulfuric acid and 0.3 ml 5% sodium tungstate, and glutamate formed was determined by using *Leuconostoc mesenteroides* ATCC 9135 by the method of Tamura et al. [17].

2.4. *Streptomyces strain*

The strain of Streptomyces employed in the production of gabaculine, which was isolated from a soil from Tohoku District in Japan, was found to be closely related to *Streptomyces toyocaensis* and designated as *St. toyocaensis* No. 1039 according to Shirling and Gottlieb [18,19].

3. Results and discussion

3.1. *Isolation of gabaculine*

Streptomyces toyocaensis No. 1039 was grown aerobically in a medium containing 2% soluble starch, 1% glucose, 1.5% Pharmamedia (Traders Oil Mill Co. USA), 2% corn steep liquor (Corn Products Co. USA) and 1% Lab-Lemco beef extract (Oxoid Ltd, England) in a 50-l fermentor for 96 h at 27°C. To the culture filtrate (21 liters), 1% of active carbon (Wako Pure Chemical, Japan) was added, at pH 2.5 and after stirring for 30 min the mixture was filtered. The resulting filtrate was adjusted to pH 7.0 with sodium hydroxide and applied to a column (12 × 71 cm) of Diaion SK-1B (H form) (Nippon Rensui, Japan). The column was washed with 17 liters water and then eluted with 0.5 N ammonium hydroxide. The active eluate (30 liters) was concentrated to 900 ml under reduced pressure and then added to 8 liters 80% methanol. The precipitate formed was removed by filtration. The filtrate was concentrated to 110 ml under reduced pressure, adjusted to pH 4.0 with acetic acid and then applied to a column (3 × 35 cm) of Amberlite IR-120 (H form) (Rohm and Haas USA) equilibrated with 0.2 M sodium acetate buffer, pH 4.0. The column was eluted with 4 liters 0.2 M sodium acetate buffer, pH 4.2 and then with 0.2 M sodium acetate buffer, pH 4.5. The eluate containing gabaculine (15.6 liters) was evaporated to dryness under reduced pressure, yielding 8.1 g of brownish powder. This powder was submitted to chromatography in a cellulose column (10 × 65 cm) equilibrated with *n*-butanol/water (100:7). The column was developed with the same solvent and the active eluate (21.5 liters) was concentrated to 150 ml under reduced pressure. The resulting precipitate was collected by filtration, dissolved in a small volume of water and then lyophilized, giving 102 mg purified gabaculine.

The purified gabaculine was homogeneous as judged by thin-layer chromatography on silica-gel plates (60F-254, E. Merck) in ethanol/concentrated ammonia/water (6:2:1) (R_f 0.65) and on cellulose plates (F, E. Merck) in *n*-butanol/acetic acid/water (3:1:1) (R_F 0.53).

3.2. *Inhibitory activity*

As shown in fig.1, gabaculine was inhibitory to GABA aminotransferase from two sources, beef brain mitochondria and *Ps. ovalis*. Concentrations required for 50% inhibition were 1.1 μM for beef brain mitochondrial enzyme and 0.7 μM for *Ps. ovalis* enzyme, respectively. The enzyme contained in the cytosolic fraction of beef brain was also inhibited by gabaculine to a similar extent (data not shown). The inhibition was competitive with respect to GABA and uncompetitive with respect to α-ketoglutarate (figs 2 and 3). The K_i-values were calculated to be 0.80 μM for mitochondrial enzyme and 0.37 μM for bacterial enzyme, respectively. Under these conditions, K_m-values for GABA and α-ketoglutarate were 21 mM and 5 mM with beef brain mitochondrial enzyme and 15 mM and 7.1 mM with bacterial enzyme, respectively.

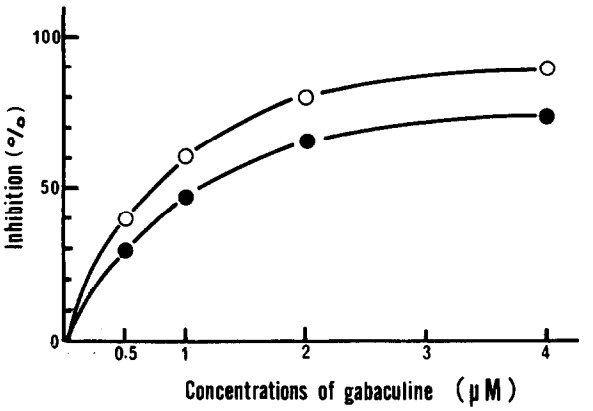

Fig.1. Inhibition by gabaculine of GABA aminotransferase from beef brain and *Pseudomonas ovalis*. Enzyme activity was determined as described in Materials and methods. The values for control were 0.25 mmol/h/mg protein for brain enzyme and 0.40 mmol/h/mg protein for bacterial enzyme, respectively. (-●-) Beef brain mitochondrial enzyme. (-○-) *Ps. ovalis* enzyme.

As shown above, gabaculine is a potent inhibitor of GABA aminotransferase. When administered systemically to mice at a dose of 50 mg/kg, gabaculine produces an increase of approximately 7-fold in brain GABA-level 8 h after administration [20], indicating that this compound is one of the metabolic inhibitors useful in the studies of physiological role of GABA in central nervous system mechanism.

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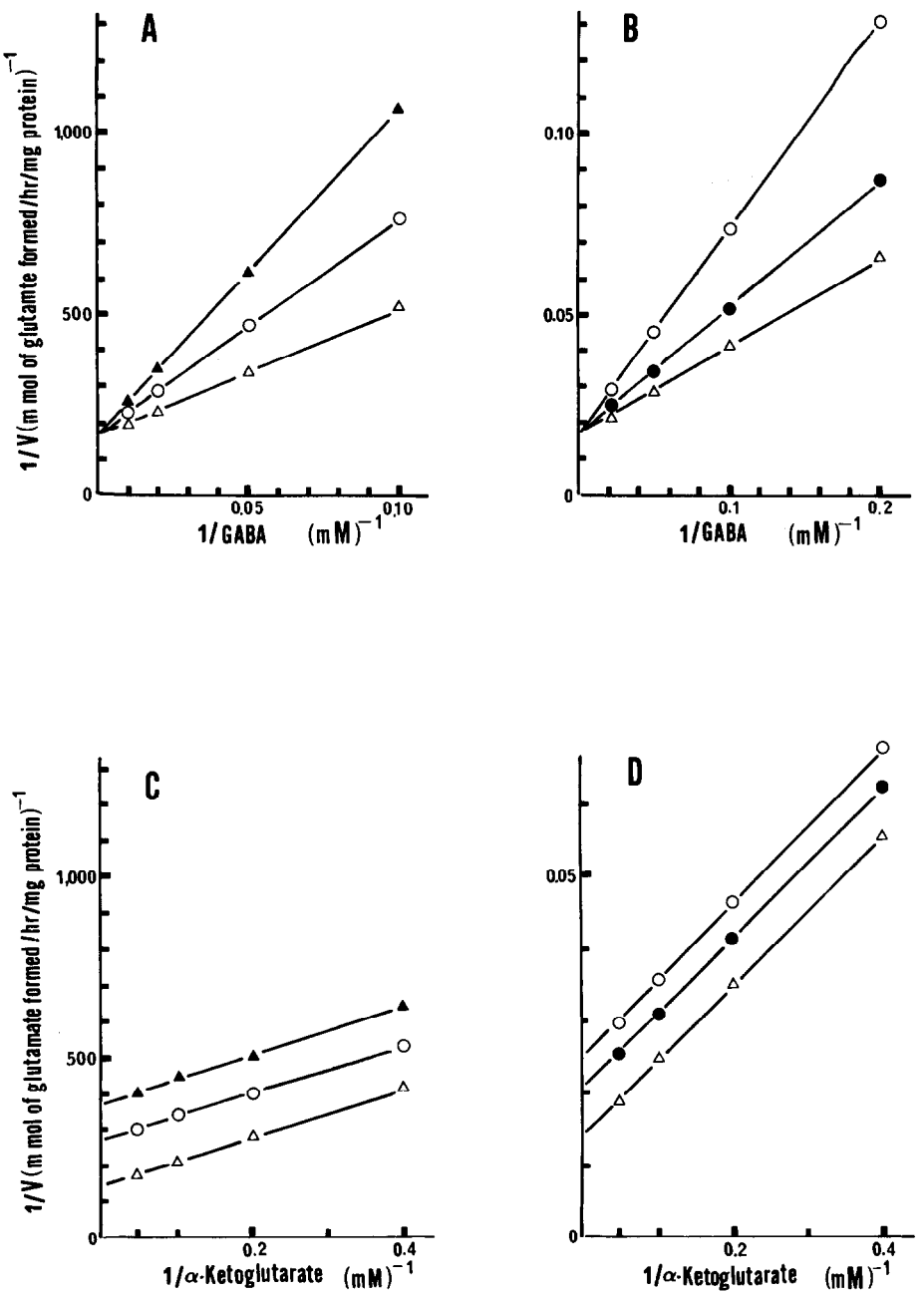

Fig.2. Double reciprocal plots of the inhibition of GABA aminotransferase by gabaculine. Experiments were carried out as described in Materials and methods, except that concentrations of GABA (in A and B) and α-ketoglutarate (in C and D) were varied as indicated. Enzymes used were beef brain mitochondria in (A) and (C) and *Ps. ovalis* cell-free extracts in (B) and (D). (-△-) Control, (-●-) 0.25 μM gabaculine, (-○-) 0.5 μM gabaculine, (-▲-) 1.0 μM gabaculine.

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